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(21) International Application Number: PCT/US98/02049 (22) International Filing Date: 4 February 1998 (04.02.98) (30) Priority Data: 60/037,166 4 February 1997 (04.02.97) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/037,166 (CIP) Filed on 4 February 1997 (04.02.97) (71) Applicant (for all designated States except US): UNIVERSITY OF SOUTHERN CALIFORNIA [US/US]; Suite 313, 3716 South Hope Street, Los Angeles, CA 90007-4344 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): RODGERS, Kathleen, E. [US/US]; 4403 Galeano Street, Long Beach, CA 90815 (US). diZEREGA, Gere [US/US]; 1270 Hillcrest Avenue, Pasadena, CA 91106 (US). (74) Agent: HARPER, David, S.; McDonnell Boehnen Hulbert & Berghoff, 300 South Wacker Drive, Chicago, IL 60606 (US).	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW. ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>	
(54) Title: METHOD FOR ACCELERATING HEALING OF THERMAL INJURIES (57) Abstract The present invention provides methods and kits for accelerating thermal wound healing in humans, by applying an effective amount of angiotensinogen, angiotensin I (AI), AI analogues, AI fragments and analogues thereof, angiotensin II (AII), AII analogues, AII fragments or analogues thereof or AII AT ₂ type 2 receptor agonists to the thermal wound.		

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METHOD FOR ACCELERATING HEALING OF THERMAL INJURIES

5 Cross Reference

This application is a Continuation of U.S. Provisional Application No. 60/037,166 filed February 4, 1997.

Field of the Invention

10 This invention relates to methods for use in accelerating the healing of human wounds caused by thermal injuries and methods for use in accelerating growth factor release, neovascularization, re-epithelialization and extracellular matrix production at the site of a human thermal injury wound.

15 Background of the Invention

Burns are a major source of morbidity each year. More than 2 million people are thermally injured annually, of which 70,000 to 108,000 are hospitalized, and 6500 to 12,000 of those patients die as a result of their burn injuries. (Rodgers et al., *J. Burn Care Rehabil.* 18:381-388 (1997), hereby incorporated by reference in its entirety).

20 Before re-epithelialization is performed after severe thermal injury, the patient is susceptible to bacterial infection and dehydration because of fluid loss. (DeCherney et al., *J. Burn Care Rehabil.* 18:292-298 (1997); hereby incorporated by reference in its entirety).

Thermal injury in mammalian tissue results in tissue disruption and coagulation
25 of the microvasculature at the wound face (U.S. Patent Application No. 5,629,292;

hereby incorporated by reference in its entirety). Repair of such tissue represents an orderly, controlled cellular response to injury. All soft tissue wounds, regardless of size, heal in a similar manner. Tissue growth and repair are biologic systems wherein cellular proliferation and angiogenesis occur in the presence of an oxygen gradient.

5 The sequential morphological and structural changes that occur during tissue repair have been characterized in great detail and have in some instances been quantified (Hunt, T.K. et al., in *The surgical wound*, pp. 1-18, ed. F. Dineen & G. Hildrick-Smith (Lea & Febiger, Philadelphia: 1981); hereby incorporated by reference in its entirety).

The cellular morphology of a wound consists of three distinct zones (U.S. Patent Application No. 5,629,292). The central avascular wound space is oxygen deficient, acidotic and hypercarbic, and has high lactate levels. Adjacent to the wound space is a gradient zone of local anemia (ischemia) that is populated by dividing fibroblasts. Behind the leading zone is an area of active collagen synthesis characterized by mature fibroblasts and numerous newly-formed capillaries (i.e.,

15 neovascularization). While this new blood vessel growth (angiogenesis) is necessary for the healing of wound tissue, angiogenic agents are in general unable to fulfill the long-felt need of providing the additional biosynthetic requirements of tissue repair. Despite the need for more rapid healing thermal injuries, to date there has been only limited success in accelerating wound healing with pharmacological agents.

20

Summary of the Invention

The present invention provides methods for accelerating thermal wound healing in humans, by applying an effective amount of angiotensinogen, angiotensin I (AI), AI analogues, AI fragments and analogues thereof, angiotensin II (AII), AII analogues, AII

fragments or analogues thereof or AII AT₂ type 2 receptor agonists to the thermal wound.

In a further aspect, the present invention provides kits for accelerating thermal wound healing in humans, wherein the kits comprise an effective amount of
5 angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments or analogues thereof or AII AT₂ type 2 receptor agonists for healing a thermal wound, and instructions for using the amount effective of active agent as a therapeutic. In a preferred embodiment, the kit further comprises a pharmaceutically acceptable carrier. In another preferred embodiment, the kit further
10 comprises a means for delivery of the active agent to a human, including, but not limited to, bandages, wound dressings, aerosol sprays and lipid foams.

Brief Description of the Figures

FIG. 1 is a bar graph representation of the percentage of burns healed as a
15 function of time post-burn.

FIG. 2 is a line graph representation of the area of the burn as a function of days post burn.

FIG. 3 is a bar graph representation of the number of blood vessels in the burn section at various times after burn injury

20 FIG. 4 is a bar graph representation of the number of blood vessels in the burn field at various times after burn injury.

FIG. 5 is a bar graph representation of the number of proliferating cells at the edge of the burn as a function of time post-burn.

FIG 6 is a bar graph representation of the number of proliferating cells in control burns and treated burns as a function of time.

FIG. 7 is a bar graph representation of the number of cyclin positive cells in the hair follicles at the edge of the burn after treatment with various AII analogues.

5 FIG. 8 is a bar graph representation of the number of cyclin positive cells in the hair follicles at the burn site after treatment with various AII analogues.

FIG. 9 is a bar graph representation of the number of vascular channels at the site of thermal injury 7 days after treatment with various AII analogues.

A more complete appreciation of the invention and many of the attendant
10 advantages thereof will more readily be understood by reference to the following detailed description when considered in connection with the accompanying drawings.

Detailed Description of the Preferred Embodiment

All cited patents, patent applications and references are hereby incorporated by
15 reference in their entirety.

Pursuant to the present invention, accelerating the healing of thermal injuries in humans is promoted through the use of a method comprising administering an effective amount of angiotensinogen, angiotensin I (AI), AI analogues, AI fragments and analogues thereof, angiotensin II (AII), AII analogues, AII fragments or analogues
20 thereof or AII AT₂ type 2 receptor agonists. In addition to peptide agents, various nonpeptidic agents (e.g., peptidomimetics) having the requisite AT₂ agonist activity are further contemplated for use in accordance with the present invention.

U.S. Patent No. 5,015,629 to DiZerega (the entire disclosure of which is hereby incorporated by reference) describes a method for increasing the rate of healing of

wound tissue, comprising the application to such tissue of angiotensin II (AII) in an amount that is sufficient for said increase. The application of AII to wound tissue significantly increases the rate of wound healing, leading to a more rapid re-epithelialization and tissue repair. The term AII refers to an octapeptide present in humans and other species having the sequence Asp-Arg-Val-Tyr-Ile-His-Pro-Phe [SEQ ID NO:1]. The biological formation of angiotensin is initiated by the action of renin on the plasma substrate angiotensinogen (*Circulation Research* 60:786-790 (1987); Clouston et al., *Genomics* 2:240-248 (1988); Kageyama et al., *Biochemistry* 23:3603-3609; Ohkubo et al., *Proc. Natl. Acad. Sci.* 80:2196-2200 (1983); all references hereby incorporated in their entirety). The substance so formed is a decapeptide called angiotensin I (AI) which is converted to AII by the converting enzyme angiotensinase which removes the C-terminal His-Leu residues from AI [DNA SEQ ID:38]. AII is a known pressor agent and is commercially available.

Studies have shown that AII increases mitogenesis and chemotaxis in cultured cells that are involved in wound repair, and also increases their release of growth factors and production of extracellular matrices. (diZerega, U.S. Patent No. 5,015,629; Dzau et. al., *J. Mol. Cell. Cardiol.* 21:S7 (Supp III) 1989; Berk et. al., *Hypertension* 13:305-14 (1989); Kawahara, et al., *BBRC* 150:52-9 (1988); Naftilan, et al., *J. Clin. Invest.* 83:1419-23 (1989); Taubman et al., *J. Biol. Chem.* 264:526-530 (1989); Nakahara, et al., *BBRC* 184:811-8 (1992); Stouffer and Owens, *Circ. Res.* 70:820 (1992); Wolf, et al., *Am. J. Pathol.* 140:95-107 (1992); Bell and Madri, *Am. J. Pathol.* 137:7-12 (1990). In addition, AII was shown to be angiogenic in rabbit corneal eye and chick chorioallantoic membrane models (Fernandez, et al., *J. Lab. Clin. Med.* 105:141 (1985); LeNoble, et al., *Eur. J. Pharmacol.* 195:305-6 (1991).

AII may also accelerate wound repair through the generation of growth factors at the site of injury. Recent studies showed that AII increased neointima formation in the carotid artery and aorta after injury (Powell, et al., *Science* 245:186-8 (1989); Powell, et al., *J. Cardiovasc. Pharmacol.* 16:S42-9 (1991); Capron, et al., *J. Cardiovasc. Pharmacol.* 18:207-11 (1991); Osterriedes, et al., *Hypertension* 18(Supp) 1160-64 (1991); Daemen et al., *Circ. Res.* 68:450-6 (1991). AII was also shown to act as a mitogen for smooth muscle cells, fibroblasts and endothelial cells (Schelling, et al., *J. Cell. Physiol.* 98:503-13 (1979); Campbell-Boswell and Robertson, *Exp. Mol. Pathol.* 35:265-76 (1981); Emmett, et al., *J. Cell. Biol.* 103:171A (1986); Paquet, et al., *J. Hyperten.* 8:565-72 (1990); Dzau, et al., *supra*). Studies have further demonstrated that AII increases the release of growth factors of various types, including PDGF, heparin-binding EGF and transforming growth factor-beta (TGF- β), and growth-related proto-oncogenes from smooth muscle cells, endothelial cells and cardiac fibroblasts (Kawahara, et al. (1988), *supra*; Naftilan, *J. Cardiovas. Pharmacol.* 20:S37-40 (1992); Naftilan, et al., (1989), *supra*; Taubman, et al. (1989), *supra*; Nakahara, et al. (1992), *supra*; Temizer, et al. (1992), *supra*; Gibbons, et al., *J. Clin. Inv.* 90:456-61 (1992); Bell, et al., *J. Clin. Inv.* 89:315-20 (1992); Stouffer and Owens (1992), *supra*). Therefore, it is conceivable that AII acts to accelerate wound repair through increasing the levels of these growth factors in wound tissue. Additionally, AII was shown to stimulate collagen synthesis thereby suggesting a role for this factor in extracellular matrix formation (Wolf, et al., *Cell. Reg.* 2:219-27 (1991); Wolf, et al., (1992), *supra*; Zhou, et al., *FASEB. J.* 6:A1914 (1992). Wound repair also involves chemotaxis of the necessary cell types into the wound bed and AII was also shown to induce the

migration of endothelial cells and smooth muscle cells in vitro (Bell and Madri (1990), *supra*).

Many studies have focused upon AII(1-7) (AII residues 1-7) or other fragments of AII to evaluate their activity. AII(1-7) elicits some, but not the full range of effects elicited by AII (Pfeilschifter, et al., *Eur. J. Pharmacol.* 225:57-62 (1992); Jaiswal, et al., *Hypertension* 19(Supp. II):II-49-II-55 (1992); Edwards and Stack, *J. Pharmacol. Exper. Ther.* 266:506-510 (1993); Jaiswal, et al., *J. Pharmacol. Exper. Ther.* 265:664-673 (1991); Jaiswal, et al., *Hypertension* 17:1115-1120 (1991); Portsi, et al., *Br. J. Pharmacol.* 111:652-654 (1994).

The effect of AII receptors and AII receptor agonists has been examined in two experimental models of vascular injury and repair which suggest that both AII receptor subtypes (AT₁ and AT₂) play a role in wound healing (Janiak et al., *Hypertension* 20:737-45 (1992); Prescott, et al., *Am. J. Pathol.* 139:1291-1296 (1991); Kauffman, et al., *Life Sci.* 49:223-228 (1991); Viswanathan, et al., *Peptides* 13:783-786 (1992); Kimura, et al., *BBRC* 187:1083-1090 (1992). Additionally, AII and angiotensin III analogs and fragments thereof in tissue repair have been shown to be effective in tissue repair. (U.S. Patent No. 5,629,292; International Application No. WO 95/08565; International Application WO 95/08337; International Application No. WO 96/39164; all references hereby incorporated in their entirety.

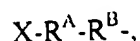
While the preceding studies suggest that AII and other AII receptor agonists may accelerate wound repair, no practical way to accelerate healing of thermal injuries in humans has been developed.

The active angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments or analogues thereof or AII AT₂ type 2

receptor agonists of particular interest in accordance with the present invention are characterized as comprising a sequence consisting of at least three contiguous amino acids of groups R^1 - R^8 in the sequence of general formula I



5 in which R^1 and R^2 together form a group of formula



wherein X is H or a one to three peptide group and a peptide bond between R^A and R^B is labile to aminopeptidase A cleavage;

R^3 is selected from the group consisting of Val, Ala, Leu, norLeu, Ile, Gly, Pro, Aib, Acpc and Tyr;

10 R^4 is selected from the group consisting of Tyr, Tyr(PO_3)₂, Thr, Ser, homoSer and azaTyr;

R^5 is selected from the group consisting of Ile, Ala, Leu, norLeu, Val and Gly;

15 R^6 is His, Arg or 6-NH₂-Phe;

R^7 is Pro or Ala; and

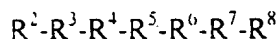
R^8 is selected from the group consisting of Phe, Phe(Br), Ile and Tyr, excluding sequences including R^4 as a terminal Tyr group.

Compounds falling within the category of AT2 agonists useful in the practice of the invention include the AII analogues set forth above subject to the restriction that R^6 is p-NH₂-Phe.

20 In one class of preferred embodiments, R^A is suitably selected from Asp, Glu, Asn, Acpc (1-aminocyclopentane carboxylic acid), Ala, Me₂Gly, Pro, Bet, Glu(NH₂), Gly, Asp(NH₂) and Suc. R^B is suitably selected from Arg, Lys, Ala, Orn, Ser(Ac), Sar,

D-Arg and D-Lys. Particularly preferred combinations for R^A and R^B are Asp-Arg, Asp-Lys, Glu-Arg and Glu-Lys. Particularly preferred embodiments of this class include the following: AII, AIII or AII(2-8), Arg-Val-Tyr-Ile-His-Pro-Phe [SEQ ID NO:2]; AII(3-8), also known as des1-AIII or AIV, Val-Tyr-Ile-His-Pro-Phe [SEQ ID NO:3]; AII(1-7), Asp-Arg-Val-Tyr-Ile-His-Pro [SEQ ID NO:4]; AII(2-7), Arg-Val-Tyr-Ile-His-Pro [SEQ ID NO:5]; AII(3-7), Val-Tyr-Ile-His-Pro [SEQ ID NO:6]; AII(5-8), Ile-His-Pro-Phe [SEQ ID NO:7]; AII(1-6), Asp-Arg-Val-Tyr-Ile-His [SEQ ID NO:8]; AII(1-5), Asp-Arg-Val-Tyr-Ile [SEQ ID NO:9]; AII(1-4), Asp-Arg-Val-Tyr [SEQ ID NO:10]; and AII(1-3), Asp-Arg-Val [SEQ ID NO:11]. Other preferred
 10 embodiments include: Arg-norLeu-Tyr-Ile-His-Pro-Phe [SEQ ID NO:12] and Arg-Val-Tyr-norLeu-His-Pro-Phe [SEQ ID NO:13]. Still another preferred embodiment encompassed within the scope of the invention is a peptide having the sequence Asp-Arg-Pro-Tyr-Ile-His-Pro-Phe [SEQ ID NO:31]. AII(6-8), His-Pro-Phe [SEQ ID NO:14] and AII(4-8), Tyr-Ile-His-Pro-Phe [SEQ ID NO:15] were also tested and found
 15 not to be effective.

Another class of compounds of particular interest in accordance with the present invention are those of the general formula II



in which R² is selected from the group consisting of H, Arg, Lys, Ala, Orn, Ser(Ac), Sar, D-Arg and D-Lys;
 20

R³ is selected from the group consisting of Val, Ala, Leu, norLeu, Ile, Gly, Pro, Aib, Acpc and Tyr;

R^4 is selected from the group consisting of Tyr, Tyr(PO₃)₂, Thr, Ser, homoSer and azaTyr;

R^5 is selected from the group consisting of Ile, Ala, Leu, norLeu, Val and Gly;

5 R^6 is His, Arg or 6-NH₂-Phe;

R^7 is Pro or Ala; and

R^8 is selected from the group consisting of Phe, Phe(Br), Ile and Tyr.

A particularly preferred subclass of the compounds of general formula II has the formula

10 R^2 - R^3 -Tyr- R^5 -His-Pro-Phe [SEQ ID NO:16]

wherein R^2 , R^3 and R^5 are as previously defined. Particularly preferred is angiotensin III of the formula Arg-Val-Tyr-Ile-His-Pro-Phe [SEQ ID NO:2]. Other preferred compounds include peptides having the structures Arg-Val-Tyr-Gly-His-Pro-Phe [SEQ ID NO:17] and Arg-Val-Tyr-Ala-His-Pro-Phe [SEQ ID NO:18]. The
15 fragment AII(4-8) was ineffective in repeated tests; this is believed to be due to the exposed tyrosine on the N-terminus.

In the above formulas, the standard three-letter abbreviations for amino acid residues are employed. In the absence of an indication to the contrary, the L-form of the amino acid is intended. Other residues are abbreviated as follows:

20

TABLE I

Abbreviation for Amino Acids

Me ² Gly	N,N-dimethylglycyl
Bet	1-carboxy-N,N,N-trimethylmethanaminium hydroxide inner salt (betaine)
Suc	Succinyl
Phe(Br)	p-bromo-L-phenylalanyl
azaTyr	aza- α' -homo-L-tyrosyl
Acpc	1-aminocyclopentane carboxylic acid
Aib	2-aminoisobutyric acid
Sar	N-methylglycyl (sarcosine)

It has been suggested that AII and its analogues adopt either a *gamma* or a *beta* turn (Regoli, et al., *Pharmacological Reviews* 26:69 (1974). In general, it is believed that neutral side chains in position R³, R⁵ and R⁷ may be involved in maintaining the appropriate distance between active groups in positions R⁴, R⁶ and R⁸ primarily responsible for binding to receptors and/or intrinsic activity. Hydrophobic side chains in positions R³, R⁵ and R⁸ may also play an important role in the whole conformation of the peptide and/or contribute to the formation of a hypothetical hydrophobic pocket.

Appropriate side chains on the amino acid in position R² may contribute to affinity of the compounds for target receptors and/or play an important role in the conformation of the peptide. For this reason, Arg and Lys are particularly preferred as R².

For purposes of the present invention, it is believed that R³ may be involved in the formation of linear or nonlinear hydrogen bonds with R⁵ (in the *gamma* turn model) or R⁶ (in the *beta* turn model). R³ would also participate in the first turn in a *beta*

antiparallel structure (which has also been proposed as a possible structure). In contrast to other positions in general formula I, it appears that beta and gamma branching are equally effective in this position. Moreover, a single hydrogen bond may be sufficient to maintain a relatively stable conformation. Accordingly, R³ may suitably be selected
5 from Val, Ala, Leu, norLeu, Ile, Gly, Pro, Aib, Acpc and Tyr.

With respect to R⁴, conformational analyses have suggested that the side chain in this position (as well as in R³ and R⁵) contribute to a hydrophobic cluster believed to be essential for occupation and stimulation of receptors. Thus, R⁴ is preferably selected from Tyr, Thr, Tyr (PO₃)₂, homoSer, Ser and azaTyr. In this position, Tyr is
10 particularly preferred as it may form a hydrogen bond with the receptor site capable of accepting a hydrogen from the phenolic hydroxyl (Regoli, et al. (1974), *supra*).

In position R⁵, an amino acid with a β aliphatic or alicyclic chain is particularly desirable. Therefore, while Gly is suitable in position R⁵, it is preferred that the amino acid in this position be selected from Ile, Ala, Leu, norLeu, Gly and Val.

15 In the angiotensinogen, angiotensin I (AI), AI analogues, AI fragments and analogues thereof, angiotensin II (AII), AII analogues, AII fragments or analogues thereof or AII AT₂ type 2 receptor agonists of particular interest in accordance with the present invention, R⁶ is His, Arg or 6-NH₂-Phe. The unique properties of the imidazole ring of histidine (e.g., ionization at physiological pH, ability to act as proton donor or
20 acceptor, aromatic character) are believed to contribute to its particular utility as R⁶. For example, conformational models suggest that His may participate in hydrogen bond formation (in the *beta* model) or in the second turn of the antiparallel structure by influencing the orientation of R⁷. Similarly, it is presently considered that R⁷ should be Pro in order to provide the most desirable orientation of R⁸. In position R⁸, both a

hydrophobic ring and an anionic carboxyl terminal appear to be particularly useful in binding of the analogues of interest to receptors; therefore, Tyr and especially Phe are preferred for purposes of the present invention.

Analogues of particular interest include the following:

5 TABLE 2

Angiotensin II Analogues

AII Analogue Name	Amino Acid Sequence	Sequence Identifier
Analogue 1	Asp-Arg-Val-Tyr-Val-His-Pro-Phe	SEQ ID NO: 19
Analogue 2	Asn-Arg-Val-Tyr-Val-His-Pro-Phe	SEQ ID NO: 20
Analogue 3	Ala-Pro-Gly-Asp-Arg-Ile-Tyr-Val-His-Pro-Phe	SEQ ID NO: 21
Analogue 4	Glu-Arg-Val-Tyr-Ile-His-Pro-Phe	SEQ ID NO: 22
Analogue 5	Asp-Lys-Val-Tyr-Ile-His-Pro-Phe	SEQ ID NO: 23
Analogue 6	Asp-Arg-Ala-Tyr-Ile-His-Pro-Phe	SEQ ID NO: 24
Analogue 7	Asp-Arg-Val-Thr-Ile-His-Pro-Phe	SEQ ID NO: 25
Analogue 8	Asp-Arg-Val-Tyr-Leu-His-Pro-Phe	SEQ ID NO: 26
Analogue 9	Asp-Arg-Val-Tyr-Ile-Arg-Pro-Phe	SEQ ID NO: 27
Analogue 10	Asp-Arg-Val-Tyr-Ile-His-Ala-Phe	SEQ ID NO: 28
Analogue 11	Asp-Arg-Val-Tyr-Ile-His-Pro-Tyr	SEQ ID NO: 29
Analogue 12	Pro-Arg-Val-Tyr-Ile-His-Pro-Phe	SEQ ID NO: 30
Analogue 13	Asp-Arg-Pro-Tyr-Ile-His-Pro-Phe	SEQ ID NO: 31
Analogue 14	Asp-Arg-Val-Tyr(Po ₃) ₂ -Ile-His-Pro-Phe	SEQ ID NO: 32
Analogue 15	Asp-Arg-norLeu-Tyr-Ile-His-Pro-Phe	SEQ ID NO: 33
Analogue 16	Asp-Arg-Val-Tyr-norLeu-His-Pro-Phe	SEQ ID NO: 34
Analogue 17	Asp-Arg-Val-homoSer-Tyr-Ile-His-Pro-Phe	SEQ ID NO: 35

The polypeptides of the instant invention may be synthesized by any conventional method, including, but not limited to, those set forth in J. M. Stewart and
 10 J. D. Young, *Solid Phase Peptide Synthesis*, 2nd ed., Pierce Chemical Co., Rockford, Ill. (1984) and J. Meienhofer, *Hormonal Proteins and Peptides*, Vol. 2, Academic Press, New York, (1973) for solid phase synthesis and E. Schroder and K. Lubke, *The Peptides*, Vol. 1, Academic Press, New York, (1965) for solution synthesis. The disclosures of the foregoing treatises are incorporated by reference herein.

In general, these methods involve the sequential addition of protected amino acids to a growing peptide chain (U.S. Patent No. 5,693,616, herein incorporated by reference in its entirety). Normally, either the amino or carboxyl group of the first amino acid and any reactive side chain group are protected. This protected amino acid is then either attached to an inert solid support, or utilized in solution, and the next amino acid in the sequence, also suitably protected, is added under conditions amenable to formation of the amide linkage. After all the desired amino acids have been linked in the proper sequence, protecting groups and any solid support are removed to afford the crude polypeptide. The polypeptide is desalted and purified, preferably chromatographically, to yield the final product.

Preferably, peptides are synthesized according to standard solid-phase methodologies, such as may be performed on an Applied Biosystems Model 430A peptide synthesizer (Applied Biosystems, Foster City, Calif.), according to manufacturer's instructions. Other methods of synthesizing peptides or peptidomimetics, either by solid phase methodologies or in liquid phase, are well known to those skilled in the art.

In one aspect of the present invention, a method for accelerating thermal wound healing in humans is provided, which comprises applying an effective amount of angiotensinogen, angiotensin I (AI), AI analogues, AI fragments and analogues thereof, angiotensin II (AII), AII analogues, AII fragments or analogues thereof or AII AT₂ type 2 receptor agonists to the thermal wound.

The compounds of the present invention can significantly accelerate the healing of thermally injured tissue at nanomolar levels *in vivo*. For any given active agent, the optimum concentration for a given formulation may be readily determined empirically.

In general, a concentration of active agent suitable for use in accordance with the present invention ranges from about 0.1 nanograms per kilogram to about 1 milligrams per kilogram.

The compounds of the present invention may be administered by any suitable
5 route, including, but not limited to, orally, parentally, by inhalation spray, rectally, transdermally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or
10 intraperitoneally.

The angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments and analogues thereof and/or AII AT₂ type 2 receptor agonists may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (e.g., solutions, suspensions, or emulsions). The
15 compounds of the invention may be applied in a variety of solutions. Suitable solutions for use in accordance with the invention are sterile, dissolve sufficient amounts of the peptide, and are not harmful for the proposed application. In this regard, the compounds of the present invention are very stable but are hydrolyzed by strong acids and bases. The compounds of the present invention are soluble in organic solvents and
20 in aqueous solutions at pH 5-8.

The angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments and analogues thereof and/or AII AT₂ type 2 receptor agonists may be subjected to conventional pharmaceutical operations such as

sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc.

While angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments and analogues thereof and/or AII AT₂ type 2
5 receptor agonists can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more other agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions that are given at the same time or different times, or the therapeutic agents can be given as a single composition.

10 For administration, the angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments and analogues thereof and/or AII AT₂ type 2 receptor agonists are ordinarily combined with one or more adjuvants appropriate for the indicated route of administration. The compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alcanoic acids, stearic acid,
15 talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the compounds of this invention may be dissolved in saline, water, polyethylene glycol, propylene glycol, carboxymethyl cellulose colloidal solutions,
20 ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials well known in the art.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin (e.g., liniments, lotions, ointments, creams, or pastes) and drops suitable for administration to the eye, ear, or nose.

5 The dosage regimen for thermal wound healing with angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments and analogues thereof and/or AII AT₂ type 2 receptor agonists is based on a variety of factors, including the age, weight, sex, medical condition of the individual, the severity of the condition, the route of administration, and the particular compound employed.

10 Thus, the dosage regimen may vary widely, but can be determined routinely by a physician using standard methods. Dosage levels of the order of between 0.1 ng/kg and 1 mg/kg angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments and analogues thereof and/or AII AT₂ type 2 receptor agonists per body weight are useful for all methods of use disclosed herein. For

15 example, angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments and analogues thereof and/or AII AT₂ type 2 receptor agonists are administered to a patient with a partial thickness burn (i.e.: second-degree burn) to trunk, back, upper arm, upper leg of at 10 cm² in area or more, and having a total body burn <40% twice daily for between 1 and 30 days.

20 Any type of application means may be employed that permits the influx of the active agents into the thermally-injured tissue over a period of time. For example, an aqueous solution could be applied to the wound tissue through a gauze bandage or strip, or such a solution could be formulated so that a timed perfusion may be obtained (using liposomes, ointments, micelles, etc.) Methods for the production of these formulations

with the compounds of the present invention are apparent to those of ordinary skill in the art. The particular concentration of active agent employed is not critical, as the tissue-repairing effect is present even when the compounds are present in nanomolar quantities.

5 In a preferred embodiment, a matrical or micellar solution is employed with the active agent present in a concentration of at least 0.1 micrograms per milliliter. In another preferred embodiment, the active agent is present in a semi-solid polyethylene glycol polymer sold under the trademark HYDRON by Hydro Med Sciences, New Brunswick, New Jersey. In a further preferred embodiment, the active agent is present
10 in a micellar solution sold under the trade name PLURONICS F108 by BASF, Ludwigshafen, Germany. Under room temperature conditions, this solution is a liquid, but when applied to warm tissue the solution forms a gel that permits infusion of the active agent into the thermally injured tissue over a period of several days. Other preferred formulations include carboxymethyl cellulose preparations, crystalloid
15 preparations (e.g., saline, Ringer's lactate solution, phosphate-buffered saline, etc.), viscoelastics, polyethylene glycols, polypropylene glycols and wound dressings (e.g., bandages, etc.).

In another embodiment of the present invention, the angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments and
20 analogues thereof and/or AII AT₂ type 2 receptor agonist is administered topically. A suitable topical dose of active ingredient of angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments and analogues thereof and/or AII AT₂ type 2 receptor agonists is preferably between about 0.1 ng/ml and about 1 mg/ml administered twice daily. For topical administration, the active

ingredient may comprise from 0.0001% to 10% w/w, e.g., from 1% to 2% by weight of the formulation, although it may comprise as much as 10% w/w, but preferably not more than 5% w/w, and more preferably from 0.01% to 1% of the formulation.

In a further preferred embodiment, transdermal means including, but not limited to, transdermal patches may be utilized to deliver the angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII analogues, AII fragments or analogues thereof or AII AT₂ type 2 receptor agonists to the treatment site. Transdermal formulations may be prepared by incorporating the active agent in a thixotropic or gelatinous carrier including, but not limited to, a cellulose medium, e.g., methyl cellulose or hydroxyethyl cellulose, with the resulting formulation then being packed in a transdermal device adapted to be secured in dermal contact with the thermal wound of a wearer.

In a further aspect, the present invention provides kits for accelerating thermal wound healing in humans, wherein the kits comprise an effective amount of angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments or analogues thereof or AII AT₂ type 2 receptor agonists for healing a thermal wound, and instructions for using the amount effective of active agent as a therapeutic. In a preferred embodiment, the kit further comprises a pharmaceutically acceptable carrier, such as those adjuvants described above. In another preferred embodiment, the kit further comprises a means for delivery of the active agent to a human. Such devices include, but are not limited to matrical or micellar solutions, bandages, wound dressings, aerosol sprays, lipid foams, transdermal patches, topical administrative agents, polyethylene glycol polymers, carboxymethyl cellulose preparations, crystalloid preparations (e.g., saline, Ringer's lactate solution,

phosphate-buffered saline, etc.), viscoelastics, polyethylene glycols, and polypropylene glycols. The means for delivery may either be impregnated with the effective amount of angiotensinogen, AI, AI analogues, AI fragments and analogues thereof, AII, AII analogues, AII fragments or analogues thereof or AII AT₂ type 2 receptor agonists for
5 healing a thermal wound, or may be separate from the compounds, which are then applied to the means for delivery at the time of application to a thermal wound.

The kits of the invention may be used in the form of a first aid kit for thermal injuries, which would be suitable for use in settings including, but not limited to, hospitals, ambulances and other emergency vehicles, schools, and the home.

10 The invention may be better understood with reference to the accompanying Examples that are intended for purposes of illustration only and should not be construed to limit the scope of the invention, as defined in the claims appended hereto. Example Nos. 1 and 2 presented below demonstrate the general utility of the invented method for accelerating healing in thermally injured tissue. Example No. 3 discloses
15 how the method of the invention can be used to promote healing of thermally injured human tissue.

Example 1: Evaluation of AII in a Guinea Pig Burn Model

Hartley guinea pigs (500 g males) were used for this study. Four burns were
20 administered per animal. The guinea pigs were anesthetized with intramuscular ketamine/rompum and the hair on the back was removed with a thioglycollate depillatory. Four deep, partial thickness burns (50 seconds, 75 degrees C, 18 mm diameter brass rods) were placed on the back of the guinea pig. The burns were treated with one of the following: placebo (10% LV CMC) or 100 ug AII in vehicle. The

guinea pigs were bandaged with a 25 mm Hill Top Chamber covered with Tegaderm (3M). The animals were then allowed to recover and were given Bupronex analgesic as they awoke. Bupronex was given each day for 3 days after burn injury. The animals were treated daily for 10 days with placebo or 100 µg/ml AII. The guinea pigs were rebandaged on days 1, 2, 3, 4, 6, 8, and 10 after burning. On the days that the bandages were removed, the burn was gently washed with warm sterile water to remove any remaining material. As the scabs came off the burns were photographed for measurement of re-epithelialization. At a rate of one per day the guinea pigs were sacrificed and the burn sites were placed in formalin for histologic preparation and immunohistochemistry.

The ability of AII to accelerate the healing of thermal injuries was then evaluated. Administration of AII for the first 10 days after burn injury led to an acceleration in the area of the injury re-epithelialized compared to placebo-treated controls on post-burn days 18-21 (FIG. 1). In addition, the number of burns completely healed was increased after administration of AII (FIG. 2).

In order to assess the effects of AII on cell function involved in burn healing, microscopic assessment was then performed on stained sections from biopsies of burns treated either with placebo (10% CMC) or 100 µg AII for the first 10 days after injury. Each day after injury, two burns from each treatment group were placed in formalin, embedded in paraffin and sectioned for microscopic analysis.

As AII was shown to be angiogenic in other animal models, the effect of AII on the number of blood vessels in the burn section at various times after burn injury was determined. At the early time points after injury very few vessels were observed in the placebo-treated burns (FIG. 3). Treatment with AII increased the number of vessels

observed in the burn area on most days post burn up through day 14. The exception to this is on days 8 and 13, the number of vessels in both groups were the same as placebo-treated burns. On days 15-28, an occasional increase in the number of new vessels was observed (days 16, 17 and 23). However, overall the number of vessels
5 during the latter phases of healing was not increased (**FIG 4**).

Immunohistochemical analysis to determine the number of proliferating cells in hair follicles at the edge and within the area of the burn was also conducted. This was measured by staining with the antibody MIB-1 which binds to the protein cyclin (Ki-67 epitope), as described in DeCherney et al., 1997 (*supra*). Cyclin is expressed in cells
10 undergoing proliferation. Administration of AII to the burn site increased the number of proliferating cells at the edge of the burn from days 3-9 (**FIG. 5**). A second increase in cyclin-positive cells at the edge of the burn was observed on days 14 to 19 after injury. Further activity of AII was observed at the burn site (**FIG. 6**). During the first
7 days after injury, almost no proliferative activity was observed in the hair follicles at
15 the burn site. However, administration of AII significantly increased the level of proliferating cells at this early time point. In fact, at most time points examined, an increase in the number of proliferating cells in the hair follicles at the burn site was observed after AII administration.

20 **Example 2: Evaluation of AII Analogues in a Guinea Pig Burn Model**

Procurement of guinea pigs and burn treatment were as described in Example 1, except that only 2 burns were applied per animal. The animals were treated for 7 days with placebo, 100 µg/ml AII, or 100 µg/ml of an AII analogue (Table 3). Each analogue was tested on a group of six guinea pigs.

Table 3. Designation for Analogues

Name	Abbreviation	Sequence
GSD 28	Ile ⁸ -AII	DRVYIHPI
GSD 24B	Pro ³ -AII	DRPYIHPI
GSD 22A	Ala ⁴ -AII	RVYAHPF
AII(1-7)		DRVYIHP

All guinea pigs were sacrificed at day 7 after burning and the burn sites were placed in formalin for histologic preparation and immunohistochemistry as in Example 1.

The results of this study demonstrate that AII and GSD 24B elevated the number of cyclin-positive cells in the hair follicles at the edge of the burn (FIG. 7), while AII and all analogues tested elevated the number of cyclin-positive cells at the burn site (FIG. 8).

Similarly, the effect of AII and AII analogues on the number of blood vessels in the burn section at various times after burn injury was determined as in Example 1 on day 7 after injury. These results demonstrate that AII and all AII analogues tested elevated the number of vascular channels at the site of the thermal injury (FIG. 9).

Example 3: Burn Healing Clinical Study Using AII

A. INTRODUCTION:

Series II will evaluate the safety, tolerance and initial pK parameters of topical AII under conditions of use (partial thickness burns). Primary safety parameters will be: blood pressure changes and changes in laboratory values. A secondary parameter will be the effect of treatment on the rate of burn site re-epithelialization.

B. STUDY DESIGN:

This study will be designed as a prospective, randomized, parallel-group, double-blind, placebo-controlled trial comparing the effects of two concentrations of AII and placebo.

Group 1: Placebo/vehicle

Group 2: 0.001% AII/vehicle

Group 3: 0.01% AII/vehicle

C. SUMMARY OF STUDY PLAN:

A patient will participate in the double-blind treatment period for a maximum of 10 days

followed by an observation period of 11 days or until the wound is 100% re-epithelialized.

Initial pK parameters will be evaluated at one study site.

Patients who satisfy the inclusion/exclusion criteria will be eligible for enrollment. After a biopsy to confirm that burn (wound) is partial thickness, a study number will be assigned to the patient. Each patient will be prospectively randomized to one of the three treatments. Such patients who enter the study and are subsequently excluded due to burn depth or drop out will be replaced so that a total of 60 patients (20 each group) will complete the study.

D. TREATMENT:

Study Medication

The following medication will be used in this trial:

Appendix A

Group 1:

5 ml glass vial containing 2 ml of:

- 15 mM Glacial Acetic Acid, USP
- 35 mM Sodium Acetate Trihydrate, USP
- q.s. Water for Injection, USP

5

Group 2:

5 ml glass vial containing 2 ml of:

- 0.05 mg/ml Angiotensin II
- 15 mM Glacial Acetic Acid, USP
- 35 mM Sodium Acetate Trihydrate, USP
- q.s. Water for Injection, USP

10

Group 3:

5 ml glass vial containing 2 ml of:

- 0.5 mg/ml Angiotensin II
- 15 mM Glacial Acetic Acid, USP
- 35 mM Sodium Acetate Trihydrate, USP
- q.s. Water for Injection, USP

15

20 The final drug product will be formed by addition of the following diluent,
under aseptic conditions at a ratio of 1 part of treatment solution to 4 parts diluent.

Diluent:

10 ml glass vial containing 10 ml of:

- 6.25% w/v Carboxymethyl cellulose Sodium, USP in Water for Injection

25

Final Drug Concentrations Post Mixing:

- Group 1: 0% Angiotensin II
- Group 2: 0.001% Angiotensin II
- Group 3: 0.01% Angiotensin II

30

All treatments will be identical in appearance in order to maintain the blind.

Study medication must be stored at refrigerated temperatures (approximately 2°-

8°C or 36°-46°F.)

2. Randomization:

Eligible patients will be placed randomly into one of three treatment groups.

The randomization list will be computer generated. This list will be used to assign
5 treatment. Neither the principal investigator nor the patients will know to which group a
patient was assigned.

3. Dosing & Schedule:

	<u>Treatment Group</u>	<u>A.M. Dose</u>	<u>P.M. Dose</u>
10	1	Placebo	Placebo
	2	0.001%AII	0.001%AII
	3	0.01%AII	0.01%AII

4. Study Drug Administration:

Wound dressings for the burn site under evaluation will be changed a minimum of
15 twice daily, i.e., once in the morning and once in the evening during administration of
study drug (10 days). The study medication or placebo will be applied twice daily with
a gloved hand, during the morning and evening dressing change. Additional treatment
of other burned areas will be performed as per the standard ward protocol. After
cessation of the study drug administration, the dressing will be changed once daily
20 during the observation period (up to 11 additional days).

5. Determination of Dose Volume:

The volume of study medication to be applied to the burn will be determined from
the dimensions of the burn measured at the screening visit. Once the volume is

calculated, that volume will be applied twice daily throughout the 10 day treatment period. To calculate the volume, multiply the length and width of the burn to obtain the surface area in cm^2 . The surface area will be expressed to two decimal places. Multiply the surface area by 0.044 ml/cm^2 . This calculation will be rounded to one decimal place. The result is the volume that will be applied.

Appendix B

Dose Preparation:

Following determination of specific dose volume to be administered to the subject, the individual doses will be prepared by an unblinded third party. The final delivered drug product in the clinical setting will be mixed under aseptic conditions at a ratio of: 1 part (20%) Angiotensin II or Placebo solution to 4 parts (80%) CMC diluent.

Mixing directions:

Multiply the total dose volume by 0.2 = Volume of Angiotensin II or Placebo solution.
Multiply the total dose volume by 0.8 = Volume of CMC diluent.

The required amount of the respective Angiotensin II or Placebo solution and CMC diluent will be separately drawn into sterile syringes, connected via a sterile syringe connector and mixed back and forth from one syringe to the other until uniform. A minimum of 10 mixes is necessary for homogeneity. The entire contents will be transferred to one of the syringes and capped and labeled. Twenty such doses will be prepared as described for each subject for the entirety of the study and stored at refrigerated conditions (approximately $2-8^{\circ}\text{C}$ or $36-46^{\circ}\text{F}$.) On a daily basis, one syringe will be dispensed for application at the clinic visit and one will be sent home with the subject for the second daily application.

Final delivered concentrations respectively will be:

Group 1: 0% AII (placebo)

Group 2: 0.001% AII

5 Group 3: 0.01% AII

E. PATIENT SELECTION AND CLINICAL PROCEDURE:

1. Screening Evaluation

10 The screening period will be defined as the time elapsed between the patient's signing the

Informed Consent and when the study medication is initially dispensed. The maximum duration of the screening period will be 12 hours. Patients may be treated with placebo during the screening period.

15 The following procedures will be performed prior to enrollment into the doubleblind portion of the study:

- a. A signed patient informed consent will be obtained;
- b. The patient will be evaluated for fulfillment of inclusion/exclusion criteria

20 (See Appendix F for procedure on assessing burns);

- c. Pain will be assessed using visual analogue pain scale. (See appendix G).

- d. A biopsy (1 x 3 mm) of two areas of the burn will be performed. (See

Appendix H for procedures)

- e. The following data will be recorded: demographic, medical history, smoking

25 history, comprehensive physical examination and vital signs, including

height, weight, blood pressure, respiratory rate and pulse;

- f. All current medications that the patient is receiving or will have received in

the ten days prior to the initial dispensing of study medication will be listed.

Radiation therapy, corticosteroid, immunosuppressive, and chemotherapeutic agents will be prohibited as described in exclusion criteria. Any "prior medications" that the patient is still taking when study medication is dispensed will then be transferred to the concomitant medications section of the case report form;

- g. Clinical laboratory evaluation. (See Appendix C for specific laboratory tests);
- h. Date and time of the burn;
- i. Wound cultures for B streptococcus and staphylococcus will be taken after debridement and before initial dressing of the wound. Biweekly surface burn wound cultures will be obtained by the nurse and sensitivities determined if necessary;
- j. The initial surgical debridement must be performed no more than 6 hours prior to patient randomization and the date and time documented on the case report form. If blisters, they must be completely unroofed and excised to reveal perfused wound margins. If an infection is present, the patient will be treated appropriately;
- k. Photo-documentation: At least two (2) 35 mm color, photographic slides of the wound will be taken initially after debridement

2. **Inclusion Criteria:**

- a. Patients will be between the ages of 10 and 75 years old;
- b. Sex: male or female;
- c. Have a partial thickness thermal burn to trunk, back, upper arm, upper leg of at least 10 cm² in area;

d. Have a total body burn <40% and under reasonable medical judgment will be expected to successfully complete study participation;

e. Are compliant and deemed to be reliable in following study requirements.

Patients must have adequate assistance or be able to ensure adherence to the

5 study medication, treatment and dressing change schedules;

f. Have given written informed consent;

3. **Exclusion Criteria:**

a. If Investigator determines study site to be a full thickness burn:

10 b. Have a known hypersensitivity to any of the study medication components;

c. Presence of any seizure disorder;

d. Grossly underweight for height;

e. Exposure to any investigational agent or medical disease with 30 days of
15 entry into study;

f. Pregnant women or nursing mothers;

g. Previous treatment under this protocol;

h. Have malignant disease of any kind. A patient who has had a malignant
disease in the past, was treated and is currently disease-free, may be
20 considered for study entry;

i. Have an HF acid burn;

j. Have a total burn area exceeding 40% body surface area:

k. POST-debridement, have study sites which contain necrotic tissue:

l. Have current or history of connective tissue disease (i.e., Rheumatoid
25 Arthritis, Systemic Lupus Erythematosus, Fibromyalgia, etc.);

m. Patients with prior history of diabetes mellitus requiring insulin;

- n. Patients with chronic renal insufficiency if the serum creatinine during screening is 2.5 mg/dL or greater;
- o. Patients with chronic active hepatitis, cirrhosis or severe ongoing liver dysfunction, as determined from medical history, physical exam and/or liver function tests (e.g., ALT, AST > 2x upper limit of normal);
- p. Burn wound areas to head, neck, joints, hands, feet, perineum and fasciotomy sites will be excluded as study sites;
- q. Presence of beta hemolytic streptococcal infection or positive culture;
- r. Receiving hemodialysis or chronic ambulatory peritoneal dialysis (CAPD) therapy;
- s. A resting diastolic blood pressure at bed rest on admission exceeding 95 mm Hg on 3 consecutive readings at least 15 minutes apart;
- t. History of chronic hypertension or receiving anti-hypertension medication;
- u. Past radiation therapy;
- v. Current use of corticosteroid, immunosuppressive, chemotherapeutics Coumadin®;
- w. Known to be HIV positive.

4. **Patient Accrual:**

Patient accrual and the duration of study will be over a 6 month period. Sixty (60) patients will be enrolled in the study with approximately equal numbers in each treatment group.

5. **Treatment Period:**

Twice daily application of study medication will begin on day 1 and will continue daily

through and include day 10. The application of study medication will coincide with the a.m.

and p.m. dressing changes (See appendix J for Clinical Procedures in Series II).

Patients will

5 be assessed daily as follows:

- a. Assess level of pain using visual analog scale prior to administration analgesic.
- b. Pain medication administered as required and recorded.
- c. Dressing removed as needed.
- 10 d. Dimension of the burn documented and assessed for re-epithelialization;
- e. Percent of surface exudate and surface re-epithelialization of burn visually estimated and recorded.
- f. Photo-documentation of wound.
- g. Exudate removed.
- 15 h. Treatment of wound.
- i. Record blood pressure, pulse and respiration 15 and 45 minutes posttreatment.
- j. Record drug dispensing and patient compliance.
- k. Document adverse events.
- 20 l. Record concomitant medication.

The patient will complete the study when 100% re-epithelialization has occurred. If the wound has shown either no improvement or has deteriorated during 10 days of double-blind treatment, or converts to a full thickness burn, the patient will be discontinued from the study and treated as determined by the investigator.

Three patients from each dose group will have plasma levels determined. As to not unblind the study, a third party representative will select three patients from each dose group for venous blood sampling. Samples will be obtained from each of these patients pretreatment and at 15 minutes posttreatment with the first dose. All blood levels will be determined.

6. **Observation Period:**

Patient visits will continue on a daily basis until day 21 or until complete re-epithelialization has occurred. Dressings will be changed during the daily visit. Clinical assessment will be as follows:

- a. The dimension of the burn will be documented and assessed for re-epithelialization;
- b. The wound will be photo-documented;
- c. Adverse events will be documented;
- d. Concomitant medication will be reported;
- e. Vital signs will be documented (BP, pulse, respiration);
- f. Pain level will be assessed.

7. **Day of Termination:**

Patients will complete the study at day 21 or upon 100% re-epithelialization whichever comes first. The following assessments will be conducted on day 21, or the day of 100% re-epithelialization, before the patient is discharged:

- a. The dimensions of burn will be documented and assessed for re-epithelialization;
- b. Photo documentation of the burn will be performed;
- c. Adverse events will be documented;

- d. Vital signs will be documented (BP, pulse, respiration);
- e. Physical examination will be performed;
- f. Urine and blood samples will be collected for clinical laboratory analysis;
- g. Concomitant medications will be recorded;
- 5 h. Evidence of hypertrophy will be assessed;
- i. Drug dispensing retrieval log will be completed;
- j. Final patient status page will be completed in case report form.

F. STATISTICAL METHODOLOGY:

10 1. General Data Management and Statistical Procedures:

All data collected on case report forms (CRF's) will be monitored at the investigational sites and reviewed for consistency and accuracy prior to data entry. Data elements questioned during that review will be resolved via queries to the investigators. CRF's will be entered into a relational database management system incorporating data
15 validation checks. All entered fields will receive 100% QC review against original CRF's. Additional data discrepancies identified during this process will also be resolved via queries to investigators. All data corrections will be subject to 100% QC.

The results of the study will be presented utilizing descriptive statistics and making comparisons between treatment groups with respect to demographic, efficacy
20 and safety parameters. Descriptive statistics by treatment group for continuous variables will consist of sample sizes, means, standard deviations, standard errors of least squares means, medians, and minimum and maximum values. Inferential analyses for continuous variables will utilize appropriate analysis of variance (ANOVA) models. For ordered categorical variables, frequencies and percentages will be displayed, and

Cochran-Mantel-Haenszel tests adjusted for investigators will be utilized. Results of categorical parameters will be displayed as frequencies and proportions by treatment group, and analyzed using chi-square, Mantel-Haenszel or Fisher's Exact Test procedures, as appropriate. Descriptive statistics for time to re-epithelialization in particular will consist of both means and medians by treatment group along with appropriate measures of dispersion, while survival analysis techniques will be utilized for treatment group comparisons. In all cases primary emphasis will be placed on the analysis of the Intent-to-Treat (ITT) population of all patients receiving any study medication and from whom any post-baseline data is available.

Demographic analyses and summary statistics by treatment group will be presented on the following parameters at baseline; age, race, gender, height, weight, medical history, concomitant medications, vital signs, clinical laboratory parameters and dimensions of the target burn. Patient accounting by treatment group will be displayed at each visit, and the proportion of patients terminating prematurely for any reason, as well as due to adverse events and due to lack of efficacy, will be statistically compared between treatment groups. The results of the latter two analyses will be highlighted in both safety and efficacy discussions. Patient accounting will also include summary statistics on exposure to drug over time.

Multiple comparisons of each active treatment group with a placebo will be implemented utilizing Fisher's LSD procedure where appropriate to control experiment Type 1 error rates. All statistical tests will utilize two-sided p-values. Differences associated with p-values <0.05 will be declared statistically significant, while p-values >0.05 and <0.10 will be interpreted as reflecting tendencies toward statistical significance. Any treatment group differences on safety parameters, and any statistical

interactions, associated with p-values < 0.10 will be explored further. Separate analyses will be performed for patient subgroups based on race, age and gender if sample sizes permit; otherwise separate tabulations will be provided for these subgroups. All analyses will be conducted utilizing SAS® version 6.11.

5 **2. Efficacy Analysis:**

The primary efficacy parameter will be time to complete re-epithelialization of the target wound. Differences among treatment groups in this parameter will be analyzed utilizing survival analysis and allowing for correct statistical treatment of right-censored observations. The survival distribution functions will be estimated by
10 the Kaplan-Meier method. Inferential comparisons between each active treatment group and placebo of survival functions will be made utilizing nonparametric rank tests and a likelihood ratio test. The effects of other covariates such as the baseline dimension of the burn will also be tested in this model. Comparisons between each active dose group and placebo on the proportion of patients with complete re-
15 epithelialization of the target wound by day 10, day 21 and at the final follow-up will be performed utilizing both Fisher's Exact Test and Cochran-Mantel-Haenszel's test adjusted for investigators as strata. Changes in estimated burn area will also be analyzed for differences between placebo and active treatment groups utilizing appropriate analysis of variance (ANOVA) models including terms for treatment,
20 investigator, the two-way interaction, and adjusted for relevant covariates as required.

3. Safety Analysis:

Time points for the analysis of safety parameters will be at day 10, day 21 and at the final follow-up visit where data are available to make those analyses possible. The proportion of patients in each treatment group reporting adverse events will be reported

and analyzed by preferred term, by body system and by severity and relationship to study medication as assessed by the investigator. Adverse events will first be coded using the WHO dictionary to allow grouping into preferred terms and body systems. Proportions of patients terminating prematurely due to adverse events will be compared statistically. Blood chemistries will be available only at screening and termination. Summary statistics will be displayed and analyses based on mean changes from screening values, on tabulations of shifts from screening values, and on clinically significant deviations from laboratory normal ranges. Mean changes from screening values in vital signs will also be displayed and analyzed by treatment group. Pharmacokinetic data (Series II) will be reviewed with clinical parameters such as blood pressure to determine if a correlation exists.

G. REGULATORY CONSIDERATIONS:

1. Adverse Events:

Any adverse event, including both observed or volunteered problems, complaints, or symptoms, will be recorded on the Adverse Event CRF. The need to capture this information is dependent upon whether the adverse event is associated with the use of the study medication. Adverse events resulting from concurrent illnesses or reactions to concurrent medications will also be recorded. Each adverse event will be evaluated for duration, intensity and relationship with the study medication or other causes. The intensity of the adverse event will be characterized as mild, moderate or severe as follows:

MILD events are usually transient, requiring no special treatment, and do not interfere with the participant's daily activities.

MODERATE events traditionally introduce a low level of inconvenience or concern to the participant and may interfere with daily activities, but are usually ameliorated by simple therapeutic measures.

SEVERE events interrupt a participant's usual daily activity and traditionally
5 require systemic drug therapy or other treatment.

When intensity changes occur more frequently than once a day, the maximum intensity rating for the event will be listed. If the intensity category changes over a number of days, then these mini-events or changes will be recorded separately (i.e., having distinct onset days).

10 The investigator will use the criteria below as a guideline for determining the relationship of the adverse event to the study drug:

- a. A temporal relationship exists between the event and the use of the drug.
- b. Re-administration of study medication results in reappearance or worsening
of the reaction:
- 15 c. Previous experience with the suspected drug resulted in a similar reaction:
- d. The event is not related to any concomitant disease, preexisting condition,
other drug therapy, or environmental factor.

One of the following determinations will then be used to document the relationship of the adverse event to the study drug:

20 NOT RELATED

POSSIBLE

PROBABLE

Any serious and unexpected adverse event including death due to any cause, which occurs during this investigation, whether or not related to the study medication,

will be reported immediately (within 24 hours) to the investigational review board.

This telephone report or fax will be followed within 5 days by a written summary fully documenting the event in order to file a report which satisfies regulatory guidelines.

- 5 All serious and unexpected adverse events associated with the use of the study medication will be immediately reported to appropriate regulatory agencies

2. Discontinuation and Replacement of Participants:

- Any participant found to have entered the study in violation of this protocol will
10 be withdrawn from the study.

An effort will be made to determine why any participant discontinues the study prematurely. This information will be recorded on the appropriate case report form.

As stated in the informed consent, all participants will reserve the right to withdraw from the study at any time.

- 15 Any participant whose condition changes after entering the study, so that he or she no longer meets the inclusion or exclusion criteria, will be withdrawn from the study.

Any participant who requires the use of an unacceptable concomitant medication will be withdrawn from the study.

- 20 The investigator will discontinue any participant from the study if in the investigator's opinion, it is not in the participant's best interest to continue.

- When a participant is lost to follow-up, that is, fails to return to study visits, a reasonable effort will be made to contact the participant in order to determine why the participant failed to return. This information will be documented on the CRF. When a
25 participant is withdrawn from the study regardless of the cause, all evaluations required the day of termination will be performed.

Participants discontinued for serious adverse events related to study treatment will not be replaced.

The date the participant is withdrawn from the study and the reason for discontinuation will be recorded on the case report form.

5 3. **Data Reporting and Case Report Forms:**

Data reflecting participant experience with the drug under investigation will be recorded on Case Report Forms.

Case Report Forms will be signed and dated by the investigator or a designated representative and filled out in black ink. If an entry on a CRF requires change, the
10 correction will be made as follows:

- a. A single line will be drawn through the incorrect entry.
- b. The date will be entered and the change initialed. White-out or erasure on CRF's will not be permitted under any circumstances.
- c. All fields and blanks will be completed. The following abbreviations will
15 be used when values or answers cannot be provided:

NA = Not applicable; ND = Not done; UNK = Not known

Original CRF's will be completed for each participant

20 **Appendix C**

Clinical Laboratory Tests:

Admission Laboratory Tests (obtained prior to enrollment in the study):

Venous blood samples will be obtained for:

- 25 a. Hematology tests: hemoglobin, WBC, including differential and platelet count.
- b. Serum chemistries: glucose, total bilirubin, creatinine, alkaline phosphatase, BUN,

SGOT

c. Urine pregnancy test; if the patient is female and of child-bearing potential.

A subject may be entered on the basis of a negative urine test (sensitive to at least 50 mIU/ml).

5 Urinalysis; a microscopic examination of sediment for RBC and WBC will be conducted.

Appendix D**Abrasion Procedure:**

10

The abrasion will be created utilizing the Chamber-Scarification Test (Frosh et al., 1976). Each volunteer will receive 1 (one) scarified site on the mid-volar surface of each forearm. (Frosch and Kligman, Contact Dermatitis 2(6): 314-324, 1976.)

15 **Appendix E****Clinical Procedures:**

1. Prior to treatment on day one, all clinical laboratory tests performed during the screening visit will be reviewed to ascertain patient eligibility;
- 20 2. On day one, a venous blood sample from each subject will be obtained at 15 minutes posttreatment for clinical laboratory tests,
3. Abrasion procedure will be performed at the selected site.
4. The length (longest edge-to-edge measurement), width (edge-to-edge measurement taken at the widest point perpendicular to the length measurement) and
- 25 depth of the abrasion in centimeters will be recorded in CRF;
5. The dose volume will be calculated and recorded on the appropriate CRF page. This volume will be used throughout the dosing period;
6. All concomitant medication will be listed on the appropriate CRF page;

7. The following steps will be followed to ensure adequate hygiene and proper application of study medication:

Step 1. Hands will be washed thoroughly and sterile gloves will be used;

Step 2. An appropriate volume of study medication will be applied. The medication should form a continuous film covering the entire area including the margins;

Step 3. The abrasion will be covered with a clean bandage and an over wrap;

Step 4. Dressings will be changed twice daily. Study medication will be applied after examination by the clinical investigator during scheduled visits to the clinic;

Step 5. Blood pressure will be monitored 15 and 45 minutes posttreatment.

Appendix F

Assessment of Partial Thickness Burns:

Complete accuracy is not yet possible in estimating the depth of a burn injury, and thus there is no reliable method for distinguishing partial thickness (PT) burns. Evaluation of burns will be made using the following criteria based on surface characteristics of the burn:

Partial thickness (PT) burns are characteristically red and painful with blisters and subcutaneous edema. This red color blanches on pressure in the early post-burn period. Because the deepest levels of cutaneous sensitivity to pinprick lie at approximately the level of the deepest epidermal derivatives, a useful diagnostic screening test is the penetration of skin by a fine needle.

A 25 gauge sterile needle will be gently rubbed over the burn site until patient indicates presence of discomfort. If no sensation is elicited, the needle will be gently inserted through the burned skin until pain is elicited up to the hub of the needle (where

bevel ends). If no pain is elicited the needle will be withdrawn and the test site assessed for bleeding. If no bleeding occurs the test will be repeated. If bleeding occurs in the absence of pain the site will be considered as full thickness and not suitable as a study site.

- 5 Following consent of the patient, a PT burn site for study will be selected by the principal investigator. One study site per patient will be selected from the upper arm, upper leg, chest, abdomen, back or buttock areas. The site will be $> 10 \text{ cm}^2$. After initial debridement the wound will be biopsied. The biopsy will be used to confirm that the burn is a partial thickness injury.

10

Criteria for Determining Depth of Injury in Thermal Injuries of the Skin:

Differentiating second degree (partial thickness) from third degree (full thickness) burns.

15

Acute second degree burn:

The superficial epidermis, when present, shows full thickness coagulative necrosis;

20

The underlying papillary dermis and superficial reticular dermis shows degeneration and homogenization of the collagen bundles with coagulative necrosis of fibroblasts;

Occasional arterioles and medium sized veins of the superficial reticular dermis may remain patent with viable endothelial cells;

25

Adnexal structures of the skin show coagulative necrosis of the superficial hair shafts but the base of hair shafts and deep pilo-sebaceous complexes show viable epithelial cells;

The deep reticular dermis shows preservation of the collagen pattern. Scattered

fibroblasts can be identified;

Blood vessels of the deep reticular dermis are patent and show intact muscle and endothelial layers.

Acute third degree burn:

5 The superficial epidermis, when present, shows full thickness coagulative necrosis;

The adnexal structures of the skin show coagulative necrosis of the entire structures including the base of hair shafts and of pilo-sebaceous complexes deep in the reticular dermis;

10 Both the papillary dermis and superficial and deep reticular dermis show degeneration and homogenization of the collagen bundles with coagulative necrosis of most fibroblasts;

All vascular structures in the papillary and superficial reticular dermis exhibit coagulative necrosis, viable endothelial cells are not found. Rare viable medium to
15 large sized arteries of the deep papillary dermis may remain patent with viable endothelial cells.

Appendix G

Visual Analog Pain Scale:

Visual Analog Pain Scale will be used to determine the amount of pain the patient
20 is experiencing prior to administration of any analgesic before treating the burn site (Screening Period; Treatment Period, twice daily). The assessment will be made by directing the patient's attention solely to the burn site then asking the patient to point to the number on the pain scale which best represents the amount of pain emanating from the study site at that time. The pain scale will be from 0 (everyday life prior to burn) to
25 10 (most excruciating pain imaginable prior to losing consciousness).

Appendix H**Biopsy Procedure:**

The biopsy site will be aseptically prepared after debridement and soap and water washes. The two areas representative of the burn are
5 biopsied sharply in a 1 X 3-mm ellipse after injection of 1% lidocaine. The wound is sutured closed using a vertical mattress interrupted technique with a 3-0 nylon suture. The wound is dressed appropriately to await the results of the biopsy. The biopsy will be analyzed to assure the wound is partial thickness. The histologic criteria to evaluate
10 burn thickness maybe seen in Appendix F.

Appendix J**Clinical Procedures:**

1. Prior to treatment on day 1, all clinical laboratory tests performed during the screening visit will be reviewed to ascertain patient eligibility;
- 15 2. The dose volume from the screening visit measurements will be calculated and recorded on the appropriate CRF page. This volume will be used throughout the dosing period;
3. Biopsy results will be reviewed as soon as available to confirm that the burn is partial thickness. The results will be documented in the CRF.
- 20 4. The level of pain will be assessed using the visual analogue pain scale;
5. Analgesics will be administered as required;
6. The dressing will be removed;
7. Surgical debridement of the burn will be performed to remove necrotic tissue fibrin, or exudate;
- 25 8. The length (longest edge-to-edge measurement), width (edge-to-edge

measurement taken at the widest point perpendicular to the length measurement) and depth of the burn in centimeters will be determined and recorded in the CRF;

9. The study site will be photographed;
10. All concomitant medication will be listed on the appropriate CRF page;
- 5 11. The following steps will be followed once or twice daily to ensure adequate hygiene and proper application of study medication:

Step 1. Hands will be washed thoroughly and sterile gloves will be used;

Step 2. Soiled dressings will be removed and disposed of (post day-1 treatment);

10 Step 3. The entire wound will be cleaned and debrided;

Step 4. An appropriate volume of study medication will be applied. The medication should form a continuous film covering the entire burn including the margins;

Step 5. The burn will be covered with a clean bandage and an over wrap;

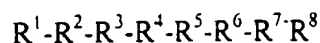
15 Step 6. The dressings will be changed twice daily. Study medication will be applied after examination by the clinical investigator during scheduled visits to the clinic;

Step 7. Blood pressure will be monitored 15 and 45 minutes posttreatment.

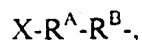
20 It is to be understood that the invention is not to be limited to the exact details of operation, or to the exact compounds, compositions, methods, procedures or embodiments shown and described, as obvious modifications and equivalents will be apparent to one skilled in the art, and the invention is therefore to be limited only by the full scope of the appended claims.

WE CLAIM:

1. A method of accelerating thermal wound healing in humans, comprising applying to the thermally injured tissue an amount effective to accelerate thermal wound healing of at least one active agent comprising a sequence consisting of at least
 5 three contiguous amino acids of groups R^1 - R^8 in the sequence of general formula I



in which R^1 and R^2 together form a group of formula



- wherein X is H or a one to three peptide group and a peptide bond between R^A
 10 and R^B is labile to aminopeptidase A cleavage;

R^3 is selected from the group consisting of Val, Ala, Leu, norLeu, Ile, Gly, Pro, Aib, Acpc and Tyr;

R^4 is selected from the group consisting of Tyr, Tyr(PO₃)₂, Thr, Ser, homoSer and azaTyr;

- 15 R^5 is selected from the group consisting of Ile, Ala, Leu, norLeu, Val and Gly;

R^6 is His, Arg or 6-NH₂-Phe;

R^7 is Pro or Ala; and

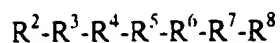
- R^8 is selected from the group consisting of Phe, Phe(Br), Ile and Tyr, excluding
 20 sequences including R^4 as a terminal Tyr group.

2. The method of claim 1 wherein the concentration of active agent is between about 0.1 ng/kg and about 1.0 mg/kg.

3. The method of claim 1 wherein the active agent is selected from the group consisting of angiotensinogen, AII, AIII, AII(2-8), SEQ ID NO:2, SEQ ID NO:3, SEQ

ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:31, and SEQ ID NO:36.

4. The method of claim 3 wherein the concentration of active agent is between
5 about 0.1 ng/kg and about 1.0 mg/kg.
5. The method of claim 1 wherein the active agent consists of angiotensin II.
6. The method of claim 5 wherein the concentration of angiotensin II is between about 0.1 ng/kg and about 1.0 mg/kg.
7. A method of accelerating thermal wound healing in humans, comprising
10 applying to the thermally injured tissue an amount effective to accelerate thermal wound healing of at least one active agent comprising a sequence consisting of at least three contiguous amino acids of groups R^2 - R^8 in the sequence of general formula II



in which R^2 is selected from the group consisting of H, Arg, Lys, Ala,
15 Orn, Ser(Ac), Sar, D-Arg and D-Lys;

R^3 is selected from the group consisting of Val, Ala, Leu, norLeu, Ile, Gly, Pro, Aib, Acpc and Tyr;

R^4 is selected from the group consisting of Tyr, Tyr(PO_3)₂, Thr, Ser, homoSer and azaTyr;

20 R^5 is selected from the group consisting of Ile, Ala, Leu, norLeu, Val and Gly;

R^6 is His, Arg or 6-NH₂-Phe;

R^7 is Pro or Ala; and

R^8 is selected from the group consisting of Phe, Phe(Br), Ile and Tyr.

8. The method of claim 7 wherein the concentration of active agent is between about 0.1 ng/kg and about 1.0 mg/kg.

9. The method of claim 7 wherein the active agent is selected from the group
5 consisting of SEQ ID NO:2, SEQ ID NO:17, and SEQ ID NO:18.

10. The method of claim 9 wherein the concentration of active agent is between about 0.1 ng/kg and about 1.0 mg/kg.

11. A kit for accelerating thermal wound healing in humans, comprising:

(a) an amount effective to accelerate accelerating thermal wound healing in
10 humans of at least one active agent comprising a sequence consisting of at least three contiguous amino acids of g groups R^1 - R^8 in the sequence of general formula I



in which R^1 and R^2 together form a group of formula



wherein X is H or a one to three peptide group and a peptide bond between R^A and R^B is labile to aminopeptidase A cleavage;

R^3 is selected from the group consisting of Val, Ala, Leu, norLeu, Ile, Gly, Pro, Aib, Acpc and Tyr;

20 R^4 is selected from the group consisting of Tyr, Tyr(PO_3)₂, Thr, Scr, homoSer and azaTyr;

R^5 is selected from the group consisting of Ile, Ala, Leu, norLeu, Val and Gly;

R^6 is His, Arg or 6-NH₂-Phe;

R^7 is Pro or Ala; and

R^8 is selected from the group consisting of Phe, Phe(Br), Ile and Tyr, excluding sequences including R^4 as a terminal Tyr group; and

(b) instructions for using the amount effective of active agent as a
5 therapeutic.

12. The kit of claim 11 further comprising a pharmaceutically acceptable carrier.

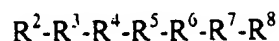
13. The kit of claim 11 further comprising a device for delivery of the active agent to a human.

14. The kit of claim 11 wherein the active agent is selected from the group
10 consisting of angiotensinogen, AII, AIII, AII(2-8), SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:31, and SEQ ID NO:36.

15. The kit of claim 11 wherein the active agent is angiotensin II.

15 16. A kit for accelerating thermal wound healing in humans, comprising:

(a) an amount effective to accelerate accelerating thermal wound healing in humans of at least one active agent comprising a sequence consisting of at least three contiguous amino acids of R^2 - R^8 in the sequence of general formula II



20 in which R^2 is selected from the group consisting of H, Arg, Lys, Ala, Orn, Ser(Ac), Sar, D-Arg and D-Lys;

R^3 is selected from the group consisting of Val, Ala, Leu, norLeu, Ile, Gly, Pro, Aib, Acpc and Tyr;

R^4 is selected from the group consisting of Tyr, Tyr(PO₃)₂, Thr, Ser, homoSer and azaTyr;

R^5 is selected from the group consisting of Ile, Ala, Leu, norLeu, Val and Gly;

5 R^6 is His, Arg or 6-NH₂-Phe;

R^7 is Pro or Ala; and

R^8 is selected from the group consisting of Phe, Phe(Br), Ile and Tyr; and

(b) instructions for using the amount effective of active agent as a
10 therapeutic.

17. The kit of claim 16 further comprising a pharmaceutically acceptable carrier.

18. The kit of claim 16 further comprising a device for delivery of the active agent to a human.

19. The kit of claim 16 wherein the active agent is selected from the group
15 consisting of SEQ ID NO:2, SEQ ID NO:17, and SEQ ID NO:18.

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FIG. 1

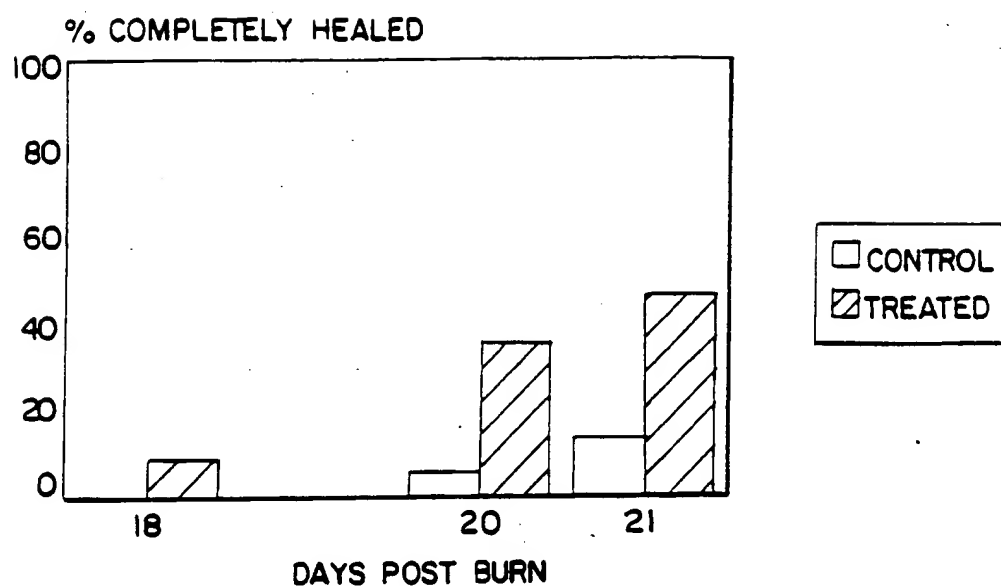
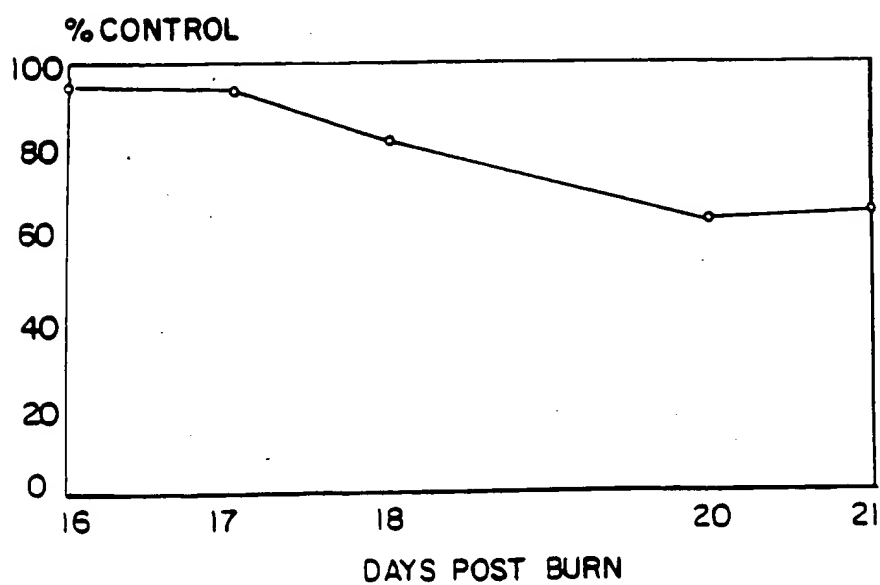


FIG. 2



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FIG. 3

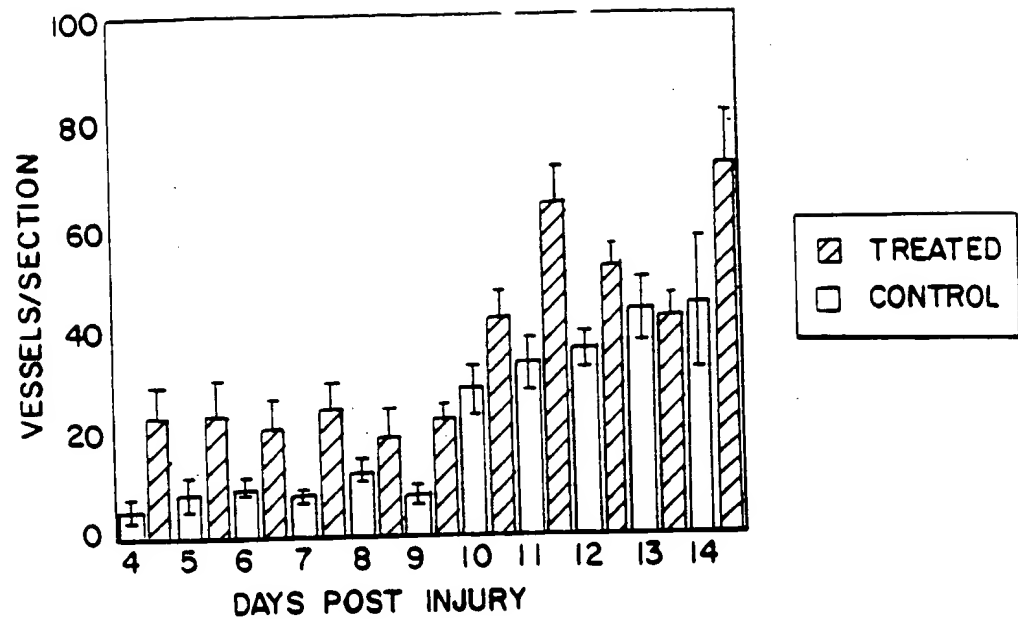
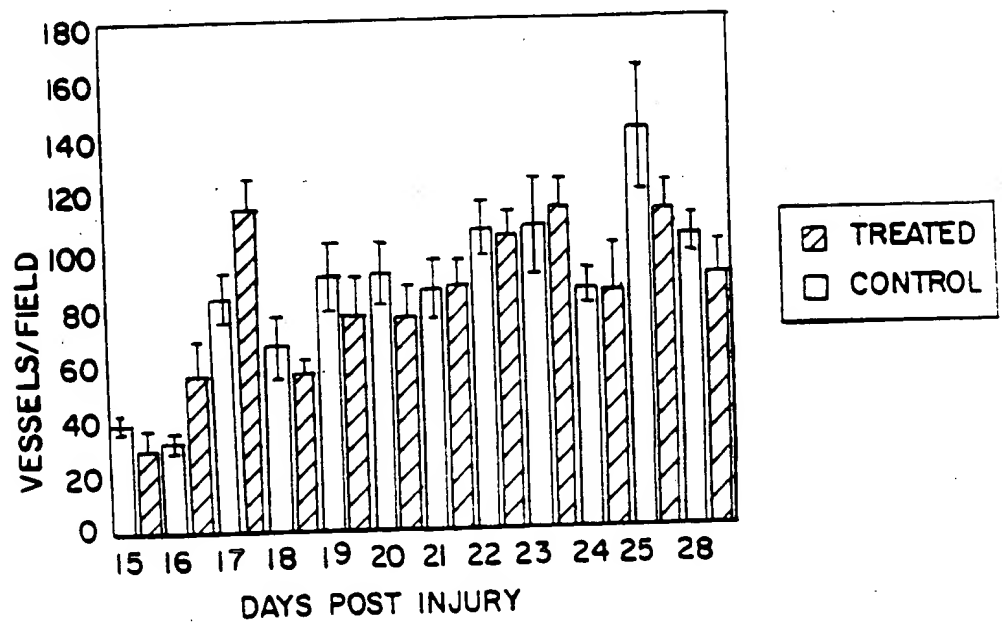


FIG. 4



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FIG. 5

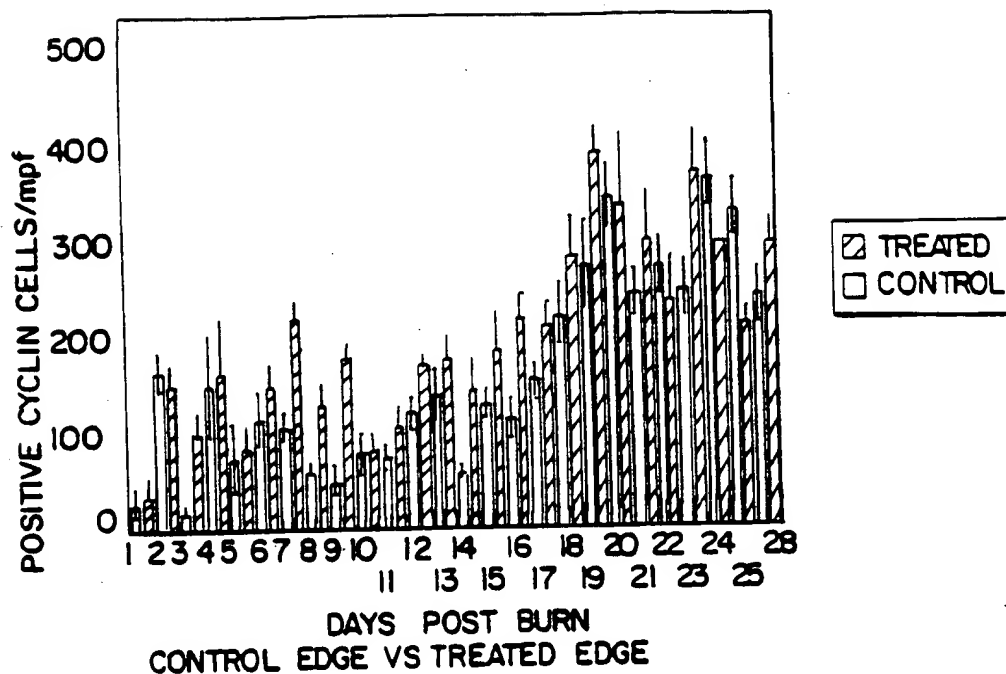
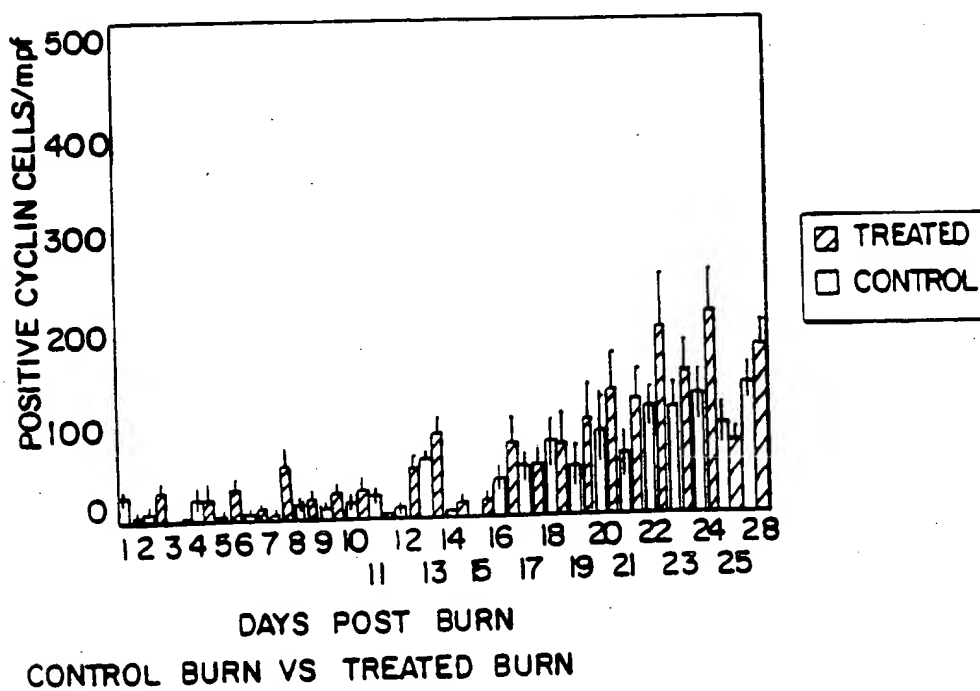


FIG. 6



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FIG. 7

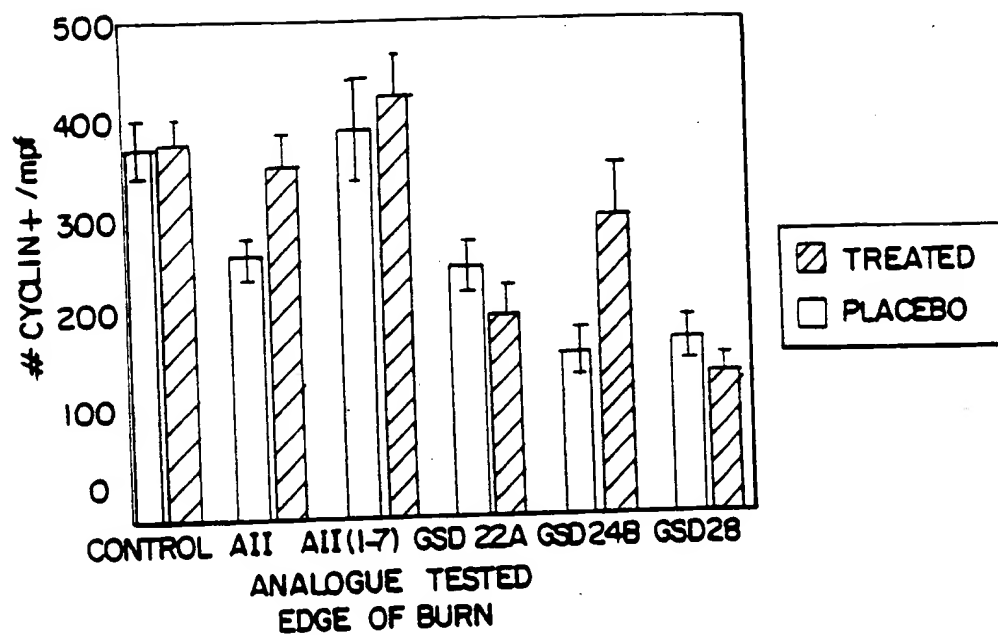
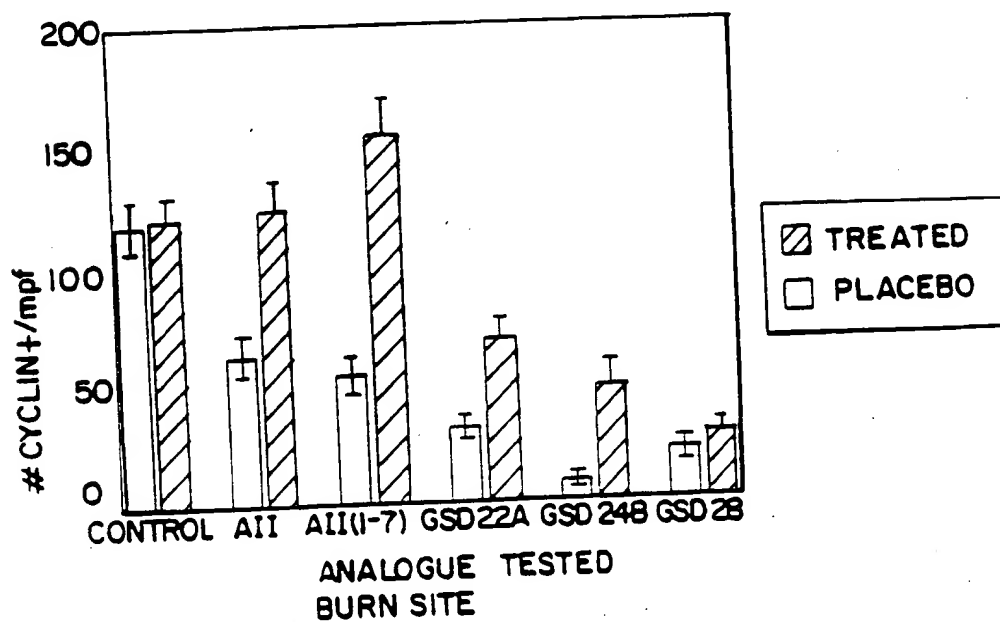
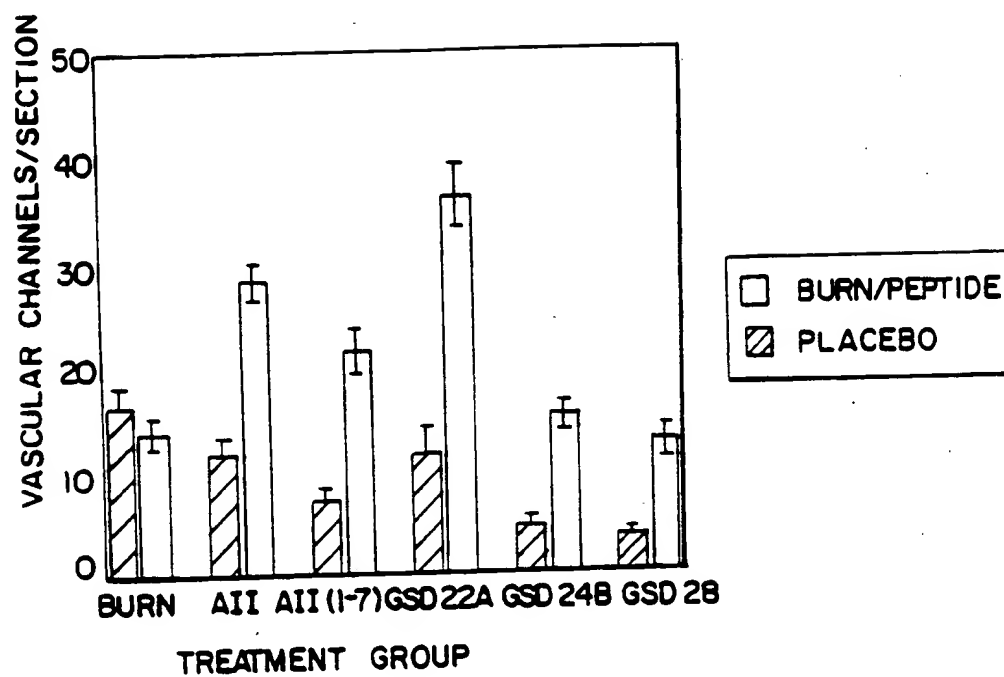


FIG. 8



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FIG. 9



INTERNATIONAL SEARCH REPORT

International application No

PCT/US 98/ 02049

Box I Observations where certain claims were found uns archable (Continuati n of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking(Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/02049

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5015629	A	14-05-1991	NONE	
WO 9508337	A	30-03-1995	AU 7730294 A CA 2172493 A EP 0730465 A JP 9502977 T US 5629292 A ZA 9407338 A	10-04-1995 30-03-1995 11-09-1996 25-03-1997 13-05-1997 10-05-1995
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(21) International Application Number: PCT/US98/02049 (22) International Filing Date: 4 February 1998 (04.02.98) (30) Priority Data: 60/037,166 4 February 1997 (04.02.97) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/037,166 (CIP) Filed on 4 February 1997 (04.02.97) (71) Applicant (for all designated States except US): UNIVERSITY OF SOUTHERN CALIFORNIA [US/US]; Suite 313, 3716 South Hope Street, Los Angeles, CA 90007-4344 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): RODGERS, Kathleen, E. [US/US]; 4403 Galeano Street, Long Beach, CA 90815 (US). diZEREGA, Gere [US/US]; 1270 Hillcrest Avenue, Pasadena, CA 91106 (US). (74) Agent: HARPER, David, S.; McDonnell Boehnen Hulbert & Berghoff, 300 South Wacker Drive, Chicago, IL 60606 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 17 September 1998 (17.09.98)
(54) Title: METHOD FOR ACCELERATING HEALING OF THERMAL INJURIES (57) Abstract The present invention provides methods and kits for accelerating thermal wound healing in humans, by applying an effective amount of angiotensinogen, angiotensin I (AI), AI analogues, AI fragments and analogues thereof, angiotensin II (AII), AII analogues, AII fragments or analogues thereof or AII AT ₂ type 2 receptor agonists to the thermal wound.		

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/02049

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K7/14 A61K38/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	US 5 015 629 A (DIZEREGA GERE S) 14 May 1991 cited in the application see the whole document ---	1-19
X	WO 95 08337 A (UNIV SOUTHERN CALIFORNIA) 30 March 1995 cited in the application see the whole document ---	1-19
X	WO 95 08565 A (UNIV SOUTHERN CALIFORNIA) 30 March 1995 cited in the application see the whole document ---	1-19
X	WO 96 14858 A (UNIV SOUTHERN CALIFORNIA) 23 May 1996 see the whole document ---	1-19
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex

Special categories of cited documents

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "Z" document member of the same patent family

Date of the actual completion of the international search

22 July 1998

Date of mailing of the international search report

30/07/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2250 HV Rijswijk
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Fax (+31-70) 340-3016

Authorized officer

Cervigni, S

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/US 98/02049

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 96 39164 A (UNIV SOUTHERN CALIFORNIA) 12 December 1996 cited in the application see the whole document -----</p>	1-19

INTERNATIONAL SEARCH REPORT

International application No

PCT/US 98/ 02049

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos. because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos. because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos. because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. l. Application No

PCT/US 98/02049

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5015629	A	14-05-1991	NONE	
WO 9508337	A	30-03-1995	AU 7730294 A	10-04-1995
			CA 2172493 A	30-03-1995
			EP 0730465 A	11-09-1996
			JP 9502977 T	25-03-1997
			US 5629292 A	13-05-1997
			ZA 9407338 A	10-05-1995
WO 9508565	A	30-03-1995	AU 7836494 A	10-04-1995
			CA 2172494 A	30-03-1995
			EP 0745091 A	04-12-1996
			JP 9503205 T	31-03-1997
			US 5716935 A	10-02-1998
			ZA 9407337 A	10-05-1995
WO 9614858	A	23-05-1996	US 5629292 A	13-05-1997
			AU 4364896 A	06-06-1996
			EP 0792158 A	03-09-1997
WO 9639164	A	12-12-1996	AU 5990796 A	24-12-1996
			EP 0828505 A	18-03-1998

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/02049

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K7/14 A61K38/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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Authorized officer

Cervigni, S

INTERNATIONAL SEARCH REPORT

Int'l. Application No.
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